ehp

LESSON:

# All Eyes on Chemical Safety Testing

**Summary** Students explore the difference between *in vitro* and *in vivo* experiments as well as the advantages and disadvantages of each. They read an article about two new *in vitro* tests for a chemical's potential to cause blindness or eye damage. Then they explore one of those tests—the bovine corneal opacity and permeability (BCOP) assay—more in depth to learn more about scientific thinking and processes.

**Lesson Type** **Extension Lesson**—this lesson extends a topic in the *EHP* article.

***EHP* Article** Ocular Safety Assays Accepted
*Environ Health Perspect* 116:A381 (2008)
http://www.ehponline.org/docs/2008/116-9/forum.html#ocul

**Objectives** By the end of this lesson, students should be able to

- list advantages and disadvantages of the *in vitro* ocular safety assays described in the article
- define *in vivo*, *in vitro*, *cornea*, *opacity*, and *permeability*
- explain specific elements of a scientific research protocol (the BCOP assay)
- identify questions that may help them better understand or critically analyze the specific elements of the protocol

**Class Time** 1 hour

**Grade Level** High school, college

**Subjects Addressed** Environmental Science, General Science

## Aligning with Standards

### SKILLS USED OR DEVELOPED

- Classification
- Communication (note taking—oral, written)
- Comprehension (listening, reading)
- Critical thinking and response
- Research
- Technological design

### SPECIFIC CONTENT ADDRESSED

- Electronic waste
- Use of graphs in presenting data

### NATIONAL SCIENCE EDUCATION STANDARDS MET

#### Science Content Standards

**Unifying Concepts and Processes Standard**

- Systems, order, and organization
- Change, constancy, and measurement
- Evidence, models, and explanation
- Form and function

**Science as Inquiry Standard**

- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

**Life Science Standards**

- Matter, energy, and organization in living systems

**Science and Technology Standards**

- Abilities of technical design
- Understanding about science and technology



National Institute of Environmental Health Sciences

**Science in Personal and Social Perspectives Standard**
- Personal and community health
- Natural and human-induced hazards
- Science and technology in local, national, and global challenges

**History and Nature of Science Standard**
- Science as a human endeavor
- Nature of scientific knowledge

## Prepping the Lesson (10–15 minutes)

### INSTRUCTIONS
1. Download the *EHP* article "Ocular Safety Assays Accepted" at http://www.ehponline.org/docs/2008/116-9/forum.html#ocul.
2. Review the Background Information, Instructions, Assessing the Lesson, and Student Instructions for this lesson.
3. Make copies of the Student Instructions and the article.
4. Make an overhead transparency of the Society of Toxicology Guiding Principles in the Use of Animals in Toxicology at the end of this lesson, or make a copy for each student.

### MATERIALS (per student)
- 1 copy of the article "Ocular Safety Assays Accepted," preferably in color
- 1 copy of the Student Instructions
- 1 copy of the Society of Toxicology Guiding Principles in the Use of Animals in Toxicology (if not used as an overhead)

### VOCABULARY
NOTE: The *EHP* article has a high reading level, and there are many words that may need to be defined for the students.

- assay
- cornea
- corrosive
- endothelium
- epithelium
- *in vitro*
- *in vivo*
- ocular
- opacity
- permeability
- protocol
- quantitative

### BACKGROUND INFORMATION
The U.S. Consumer Product Safety Commission, the U.S. Food and Drug Administration, and the U.S. Environmental Protection Agency are regulatory agencies that enforce laws or regulations enacted by the U.S. Congress to protect public health. Many of these regulations use words such as *toxic*, *hazardous substance,* and *corrosive* to describe substances that should be regulated. This lesson focuses on a recently approved assay to test a chemical for its potential to cause eye damage, primarily through corrosivity. The chemicals that would be tested for their potential to cause eye damage could have industrial, household, and cosmetic uses.

All the new chemicals created each year must be tested (the extent of the testing depends on the intended use of the chemical) to determine whether and how the chemical should be regulated. Any company that creates a chemical is required to conduct its own testing. Eventually some, but not all, the chemicals are also tested through independent research at universities and federal institutions (e.g., the National Institute of Environmental Health Sciences). This independent research is important for reducing bias and identifying the potential adverse or beneficial effects of a chemical that may be missed by the manufacturer's safety testing. This independent research plays a key role in helping regulatory agencies identify which chemicals should be regulated and how. Test results also influence how these chemicals and products are labeled.

Typically, many tests are needed to determine whether a chemical can cause cancer, hormone disruption, organ damage, birth defects, acute poisoning, or other hazards. These tests are grouped into two general categories: *in vitro* and *in vivo*. *In vitro* refers to tests conducted outside of the whole animal (often in test tubes or petri dishes), and *in vivo* refers to tests conducted in live animals.

*In vitro* and *in vivo* tests each have advantages and disadvantages, which makes them complementary to one another. *In vitro* tests are generally less expensive and take less time, lending themselves to the rapid screening technologies used to test the continuous stream of new chemicals created each year. *In vivo* tests help determine how a chemical behaves in the body (for example, whether a chemical is acutely toxic or whether it is metabolized into toxic chemicals). *In vivo* tests also allow scientists to determine how a chemical is distributed throughout the body and whether it targets a specific organ such as the brain, liver, or kidney.

*In vivo* tests are very expensive and may take years before effects are seen; therefore, *in vivo* tests are typically not conducted unless evidence from *in vitro* tests or human epidemiologic data (such as evidence of the long-term presence of chemicals in human tissue or the increased frequency of a disease) indicates a reason for concern. *In vivo* tests play a critical role in identifying toxic or hazardous substances and in identifying the biological mechanism for a toxic substance. U.S. regulators tend to assume that a chemical is not harmful unless proven otherwise (the European Union, on the other hand, has adopted the precautionary principle, which holds that protective measures should be enacted when there are reasonable grounds for concern that an agent may adversely affect health, even in the absence of scientific certainty about the risk posed by that agent). Thus, proving that a chemical is safe or unsafe requires many different kinds of evidence from both *in vitro* tests and *in vivo* tests, including a possible explanation of how a chemical causes a disease.

### RESOURCES

*Environmental Health Perspectives*, Environews by Topic page. http://ehp.niehs.nih.gov/. Choose Alternative Test Models, Animal Models

Exploratorium. Cow's Eye Dissection. An excellent eye anatomy and physiology tutorial. http://www.exploratorium.edu/learning_studio/cow_eye/

Georgetown Law Library. Animal Law Research Guide. A variety of online resources regarding animal testing and welfare. http://www.ll.georgetown.edu/guides/AnimalLaw.cfm

Mayo Clinic. Glaucoma. Information about the symptoms, risk factors, causes, and treatment of this disease. http://www.mayoclinic.com/health/glaucoma/DS00283

National Toxicology Program. *In vitro* test methods for detecting ocular corrosives and severe irritants. http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd_bcop.htm

National Toxicology Program. Testing Regulations and Guidelines. A variety of online resources regarding animal testing and welfare. http://iccvam.niehs.nih.gov/SuppDocs/SD_docs.htm

Society of Toxicology. Animals in Research. Information on the effective and humane use of laboratory animals. http://www.toxicology.org/ai/air/air.asp

U.S. Consumer Product Safety Commission. Federal Hazardous Substances Act. http://www.cpsc.gov/BUSINFO/fhsa.html

U.S. Department of Agriculture. Animal Welfare Act and Information. http://www.nal.usda.gov/awic/legislat/usdaleg1.htm

## Implementing the Lesson

### INSTRUCTIONS

1. Begin this lesson by taking a quick poll of the class on six questions provided below. The poll will engage students on the topic of using animals in scientific research and identify what students already do and don't know as well as potential emotional connections or (mis)perceptions on the topic. You can also demonstrate quick data collection and data display by generating a frequency plot or histogram for each question or by counting the number of students for each response and calculating the percentage.

   **Poll Questions**

   - How many students know the meaning of *in vitro*? (Yes, no, sort of)
   - How many students know the meaning of *in vivo*? (Yes, no, sort of)
   - How many students know what an assay is? (Yes, no, sort of)
   - How familiar are you with the topic of using animals for scientific research? (Very familiar, somewhat familiar, not familiar; very familiar may be described as someone who knows about why, when, and how animals are used, the laws concerning use of animals, and procedures used to keep animals for research, etc.)
   - What is your opinion about using animals for research? (Animals should never be used at all for research, animals should be used for research under certain circumstances, it is ok to use animals for research)
   - How useful are animals for scientific research? (Not at all useful, somewhat useful, very useful)

2. Divide the class into small groups of 2 to 3 students each to briefly discuss (about 5 minutes) their opinions of animal research and what they know about it. Facilitate as needed. For instance, provide each student 1 minute to talk freely, then allow 2 minutes at the end for the group to exchange questions, ideas, or clarifications, OR allow students to talk freely to get their ideas flowing, then regulate so one student does not monopolize the conversation. You may need to ask additional questions to prompt students (e.g., Where did you get your ideas about animal testing? What do you think it means when you see "no animal testing" on a product container?)

Some ground rules may include the following:

- be respectful by allowing others to talk and share their opinion without being interrupted
- refrain from making judgmental or demeaning comments, sounds, or gestures (like smirking or eye rolling)

3. Ask the students to define *in vitro*, *in vivo*, and *assay*. The level of explanation you provide may vary depending on your class, but some key points are as follows:
   - *In vitro* is Latin for "within glass" and generally refers to experiments conducted outside of a living whole-animal system. These experiments often use pieces of tissue (e.g., liver) or specific types of cells (e.g., cancer cells), or they can be test tube experiments using specific mixtures or manipulations of chemicals (e.g., enzyme function or protein–DNA interactions). Experiments that use "living" cell cultures—which are cells that can grow and multiply and have active biochemical-based and physical cellular processes—are sometimes called *ex vivo* because of the "living" nature of the cells, but these experiments are generally placed in the *in vitro* category.
   - *In vivo* is Latin for "within the living" and refers to research using whole organisms, which can range from bacteria, to worms, to rats, to humans.
   - An assay is a test that measures the presence, amount, or effect of a chemical.
4. Distribute the Student Instructions and the article "Ocular Safety Assays Accepted." Have students complete Steps 1 through 4. Provide reading support as needed for students. The reading level for this article is relatively high.
5. After students have completed Step 4, conclude the activity with a discussion that expands on answers given in Step 4. Share the information below with students and ask how this information affects their perspective about the use of *in vitro* versus *in vivo* assays (e.g., when might scientists need to use *in vivo* assays to identify chemicals that may affect the eye or contribute to blindness?).

   Glaucoma is the second leading cause of blindness and is caused by damage to the optic nerve. There are numerous reasons for damage to the optic nerve. The most common cause of optic nerve damage is increased pressure from fluid buildup in the eye. There are many potential causes for the buildup, one of which is that the fluid drainage angle in a person's eye is too narrow. (The drainage angle is the point between the iris and the cornea where the aqueous humor produced by the eye constantly drains off.) The angle closes when the pupil dilates so fluid cannot drain properly. Pupil dilation occurs naturally in dim light or darkness but also can occur when certain chemicals are ingested. For example, the street drug LSD (lysergic acid diethylamide) and some antihistamines and antidepressants cause pupil dilation. Eye drops used for eye exams also dilate the pupils. Although these chemicals do not explicitly cause blindness, the fact that they affect the eye is important information. The only way we can determine a chemical's effect on the body is through *in vivo* experiments, in which the chemical is metabolized by the body and distributed.
6. Some students are sensitive to the use of animals for scientific experiments, and it is important to help students understand there are very strict guidelines in the United States for the humane use of animals in experiments. For example, all experiments funded by the U.S. government must be reviewed by institutional oversight committees. The committees review the justification or explanation for the experiment and the experimental protocol to ensure all laws and the highest ethical standards are followed. If the committee is concerned about the experiment, they may request modifications to the protocol, or they may not approve the study.

   Review the Society of Toxicology Guiding Principles in the Use of Animals in Toxicology principles (included at the end of this lesson) as needed with students to clarify the extent of care and consideration given to the ethical care of animals in scientific research.
7. One final note: The statements "not tested on animals" and "no animal testing" found on many personal care products indicate that individual chemicals used in the product have been previously tested. It is unnecessary to retest a chemical already approved for a specific use.

**Notes & Helpful Hints**

- Students may want to review the Animal Welfare Act to learn more about U.S. animal protection laws. The Resources section provides a link to the text of this act as well as to the National Toxicology Program Testing Guidelines and Regulations website and the Georgetown University Law Library website, both of which provide access to many other resources related to animal law and welfare.

## Assessing the Lesson (steps not requiring teacher feedback are not listed below; see Student Instructions for complete step-by-step instructions)

**Step 1**

**a. The article describes two new ocular safety assays: the bovine corneal opacity and permeability (BCOP) test and the isolated chicken eye (ICE) test. List two advantages of having these new ocular safety assays.**

Some possible answers include:

- These assays reduce the use of live animals in testing.
- These assays reduce the potential for pain and distress in test animals by identifying the most corrosive chemicals before they are tested on live animals.
- These assays allow for testing new chemicals for their potential to cause irritation or certain types of eye injury.
- These assays increase the efficiency/speed of testing (a reasonable answer that is not articulated in the article).

**b. What must scientists do now that there is "regulatory acceptance" of the BCOP and ICE assays? How will this affect their experiments?**

The BCOP and ICE assays "must be considered as the first option for ocular safety testing."

**Step 2**

**a. Define *cornea*, *opacity*, and *permeability*. You may need to use multiple resources to help you define these terms.**

Cornea:

- The cornea is the clear outer portion of the eye that focuses light into the eye.
- The epithelium of the cornea keeps outside elements such as dust, water, and bacteria from getting into the eye.
- The epithelium of the cornea allows oxygen and nutrients from tears to pass through the eye.
- The endothelium maintains fluid balance, which helps keep the cornea clear.
- The cornea must remain clear to see well.

Opacity: The state of not allowing light to pass through.

Permeability: The state of allowing liquid or gas to pass through.

**b. Describe how the BCOP assay is used to reveal information about a chemical's potential ability to cause blindness or irritation through corrosivity (i.e., the chemical dissolves or breaks down a material). In constructing your answer, consider the name of the assay and describe what a "positive" result might look like for a corrosive chemical. Note: a "positive" result means the chemical causes an effect.**

Look for a well-constructed logical answer that includes the following elements:

- The cornea of the cow's eye is exposed to the chemical being tested.
- Changes in opacity and permeability of the cornea are measured.
- A positive result for a corrosive chemical may decrease the clarity of the cornea (increase its opacity) and/or change the permeability of the cornea. For example, a *decrease* in the permeability of the cornea could keep oxygen or nutrients from getting through normally, which would be a problem. Conversely, an *increase* in the permeability could allow dirt and bacteria to get through the cornea, which also would be a problem.

**Step 3b**

**The underlined concepts from the BCOP protocol are listed below. Write a brief description of the purpose of each concept, and add any questions you may have about that part of the protocol. Questions related to clarifying the protocol are encouraged.**

Look for answers that are clear and logical and that demonstrate that some thought was given to the purpose of the underlined element. The responses provided below are only examples of the questions that may be asked. Student questions may vary greatly; just make sure the question relates to the underlined topic and demonstrates thought and/or curiosity. A question such as "why use freshly slaughtered cattle?" is not acceptable, because it does not demonstrate thought or understanding, or advance the topic in any way.

**freshly slaughtered cattle:** The corneas used for testing should come from freshly slaughtered cattle to ensure the eye has not degraded and that the cells are able to have metabolic activity in the medium (students may not intuitively know this level of detail). Students may have a variety of questions, but those that relate to the integrity of the protocol should be encouraged. SAMPLE QUESTIONS: What does "fresh" mean? How many hours after slaughter is the cornea still viable? (For your reference, the more detailed protocol documents recommend 5 hours or less after slaughter.) Students may also wonder whether the sex, age or breed of the cattle makes a difference. (For your reference, the age of the cattle appears to be the most important factor, and it is recommended that eyes come from cattle no older than 60 months.)

**corneas free of defects:** Corneas should be free of defects before testing to ensure that any changes to the cornea occurred as a result of the chemical being tested. SAMPLE QUESTIONS: What defects should be absolutely avoided? Is there an "acceptable" number or type of defects?

**incubated at 32 ± 1°C:** Incubation temperature should be consistent; results could be compromised if it is too warm or cold or if there is a change in temperature. SAMPLE QUESTION: What happens to the eye or the results when the temperature changes?

**baseline opacity measurement is performed:** An initial measurement of corneal opacity before testing begins allows the measurement of changes resulting from exposure to the test chemical. SAMPLE QUESTIONS: How many times is the opacity test repeated for the baseline test? What is the error or variability in the opacity test? Can the initial opacity test damage the cornea? How is opacity measured? Are there different opacity tests?

**corneas are incubated horizontally for 10 ± 1 minutes:** These instructions are intended to standardize the length of exposure (10 ± 1 minutes) and ensure a position of the cornea (horizontal) that maximizes an even exposure to the test chemical. SAMPLE QUESTIONS: Why was 10 minutes chosen as the exposure time? Was it based on the amount of corrosivity produced from a known substance? Does the exposure time change for different substances, or is it always 10 minutes? (For your reference, the National Toxicology Program notes that scientists in this field have suggested changing the protocol to modify exposure times for different chemical classes, such as shampoo versus a heavy-duty industrial cleaner.)

**the epithelial surface is washed at least 3 times:** The chemical must be completely removed from the cornea. Otherwise, if it *is* corrosive, it will continue to damage the cornea, and the chemical may be categorized as being more corrosive than it really is. SAMPLE QUESTION: How is the washing done to minimize damage to the cornea from the washing action itself?

**second opacity measurement is taken:** This measurement is taken after exposure to the test chemical to determine if the chemical affected the cornea's opacity. Questions may be similar to the questions at the baseline measurement.

**incubated for . . . 2 hours prior to taking a final opacity measurement:** This measurement of opacity 2 hours later may reveal some delayed changes to the eye. SAMPLE QUESTIONS: Does this measure only delayed damage? Can the eye improve in this stage? What happens 4 hours later? 12 hours later? How long can the cornea remain "functional" once placed in the medium?

**to the anterior compartment of the corneal holder, 1 mL of sodium fluorescein (0.4% for liquids and surfactants, 0.5% for solids) is added:** This stipulates which liquid to use to test permeability, how the liquid is applied, and in what amounts. SAMPLE QUESTION: Why is a different amount used for liquids/surfactants and solids?

**the amount of dye that penetrates the cornea:** The amount of dye that passes into the cornea is compared with the control. SAMPLE QUESTIONS: Does this mean the more dye that penetrates the cornea, the more damage has occurred because the cornea's protective layers have been compromised? What other variables might affect dye absorption?

**Step 4** **Consider the entire eye and how it functions as well as the different routes of exposure for a chemical (i.e., a corrosive chemical that damages the surface layers versus a chemical that can be absorbed, ingested, or inhaled). What are some potential limitations of the BCOP assay with respect to testing "new products for their potential to cause temporary or permanent blindness, irritation, or other eye injuries"?**

Student answers may vary, but they should demonstrate logical arguments and clear communication. Answers should contrast corrosive chemicals with chemicals that enter the body in other ways. For example, one could argue that the BCOP assay may be a sufficient test for corrosivity and resulting potential for eye injury/irritation because a chemical that physically injures the cornea can also injure other parts of the eye (which, because of their location in the eye, are actually better protected than the cornea from injury caused by surface corrosion). However, the assay may be less useful for testing chemicals that can be absorbed deeper into the eye (without obvious damage to the cornea) and injure other parts of the eye important for vision. Students who have a better understanding of the absorption and distribution of chemicals in the bloodstream may recognize that some chemicals that are inhaled, absorbed, or ingested may damage vision by either distributing the chemical into the eye via blood or harming the parts of the brain that govern vision.

## Authors and Reviewers

**Author:** Stefani Hines, College of Pharmacy, University of New Mexico

**Reviewers:** Jennifer K. Campbell, Laura Hemminger, and Paul Lioy, University of Medicine & Dentistry of New Jersey; Philip M. Iannaccone, Northwestern University; Susan M. Booker, Martha M. Dimes, Erin E. Dooley, and Dorothy L. Ritter, *Environmental Health Perspectives*

**Give us your feedback!** Send comments about this lesson to ehpscienceed@niehs.nih.gov.

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STUDENT INSTRUCTIONS:

# All Eyes on Chemical Safety Testing

**Step 1** Read the article "Ocular Safety Assays Accepted" and answer the following questions.

a. The article describes two new ocular safety assays: the bovine corneal opacity and permeability (BCOP) test and the isolated chicken eye (ICE) test. List two advantages of having these new ocular safety assays.

b. What must scientists do now that there is "regulatory acceptance" of the BCOP and ICE assays? How will this affect their experiments?

**Step 2** The article states, "Several agencies require manufacturers to test new products for their potential to cause temporary or permanent blindness, irritation, or other eye injuries." Think about the words that make up the name of the BCOP assay, then answer the following questions.

a. Define *cornea*, *opacity*, and *permeability*. You may need to use multiple resources to help you define these terms.

- cornea (describe the multiple parts and functions of the cornea, tell how the cornea helps one see, and differentiate between the function of the epithelium and endothelium of the cornea):

- opacity:

- permeability:



National Institute of Environmental Health Sciences

b. Describe how the BCOP assay is used to reveal information about a chemical's potential ability to cause blindness or irritation through corrosivity (i.e., the chemical dissolves or breaks down a material). In constructing your answer, consider the name of the assay and describe what a "positive" result might look like for a corrosive chemical. Note: a "positive" result means the chemical causes an effect.

**Step 3** It is very important that companies that make and use chemicals test those chemicals for corrosivity or toxicity using a specific well-tested protocol. A well-tested protocol is more likely to provide consistent results between tests and to provide accurate information about the chemical of interest.

If different companies used different tests on the same chemical, people would argue about which test was the best one to use. Company A could say "our tests show that our chemical is safe" and Company B could say "our tests show that your chemical is not safe." If the two companies were using two completely different tests, then it would be difficult to determine which test results were correct. It would not be good for the poorly tested chemical to be released for consumer use to see if it was safe. Imagine if an untested or poorly tested chemical went into a mascara formulation, then it was discovered that the chemical was corrosive, and people who used the mascara lost their vision.

Even within a single test, many factors or variables can affect the reliability of the test. As with any experiment, investigators attempt to control the variables to be confident that the effects (or lack of effects) observed are attributable to the chemical being tested. Standardization and control of variables are important parts of scientific research.

a. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) has published the BCOP assay protocol (or instructions) on its website. This committee, made up of representatives from several U.S. government agencies, plays an important role in identifying, evaluating, and recommending chemical testing methods for use as "official" methods of chemical testing. Regulatory agencies such as the U.S. Environmental Protection Agency may then choose to accept ICCVAM's recommendations for using an assay as a standard protocol. Standardization and control of variables is an important part of the scientific process and is the primary goal of a standardized protocol such as the BCOP assay.

   Below are excerpts from the BCOP protocol. Read the excerpts and pay special attention to the parts of the protocol that are underlined, emphasizing important elements of variables controlled for in the experiment.

   "[T]he BCOP assay uses isolated corneas from the eyes of freshly slaughtered cattle. Corneas free of defects are dissected with a 2- to 3-mm [millimeter] rim of sclera remaining to assist in subsequent handling, with care taken to avoid damage to the corneal epithelium and endothelium. Isolated corneas are mounted in specially designed corneal holders that consist of anterior and posterior compartments, which interface with the epithelial and endothelial sides of the cornea, respectively. Both chambers are filled with medium [NOTE: the medium refers to a specific material used to keep the corneas from drying out; the chemical that is being tested is NOT in the medium], and the device is then incubated at 32 ± 1°C for 1 hour to allow the corneas to equilibrate with the medium and to resume normal metabolic activity.

   Following the equilibration period, fresh medium is added to both chambers, and a baseline opacity measurement is performed. Corneal opacity is measured quantitatively as the amount of light transmission through the cornea.

   Two treatment protocols are used—one for liquids and surfactants and one for solids.

Liquids are tested undiluted; surfactants are tested at a concentration of 10% in saline or deionized water. Corneas are incubated horizontally for 10 ± 1 minutes at 32 ± 1°C. The test substance is removed from the anterior compartment and the epithelial surface is washed at least 3 times. After refilling both chambers with fresh medium, a second opacity measurement is taken and the corneas are incubated again at 32 ± 1°C for 2 hours prior to taking a final opacity measurement.

Immediately after completing the final opacity measurements, corneal permeability is determined quantitatively by evaluating changes in the barrier properties of the epithelium to sodium fluorescein. To the anterior compartment of the corneal holder, 1 mL of sodium fluorescein (0.4% for liquids and surfactants, 0.5% for solids) is added. The corneas are incubated horizontally for 90 minutes at 32 ± 1°C. The amount of dye that penetrates the cornea is [measured].

A substance producing an *In Vitro* Score from 0 to 25 is considered a mild irritant, from 25.1 to 55 a moderate irritant, and from 55.1 and above a severe irritant."

b. The underlined concepts from the BCOP protocol are listed below. Write a brief description of the purpose of each concept, and add any questions you may have about that part of the protocol. Questions related to clarifying the protocol are encouraged.

freshly slaughtered cattle:

corneas free of defects:

interface with the epithelial and endothelial sides of the cornea:

incubated at 32 ± 1°C:

baseline opacity measurement is performed:

corneas are incubated horizontally for 10 ± 1 minutes:

the epithelial surface is washed at least 3 times:

measurement is taken:

incubated for . . . 2 hours prior to taking a final opacity measurement:

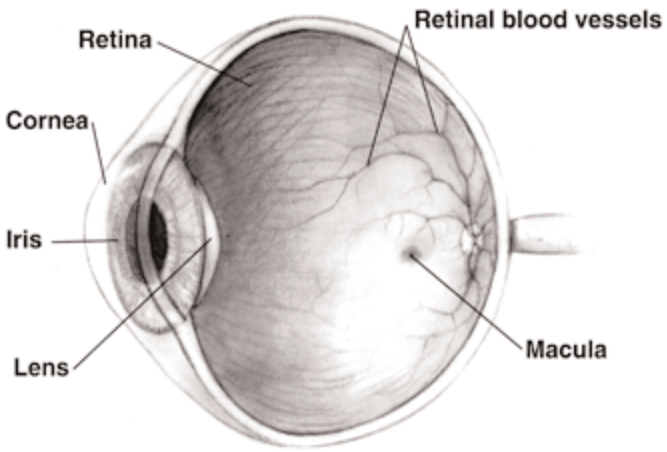
to the anterior compartment of the corneal holder, 1 mL of sodium fluorescein (0.4% for liquids and surfactants, 0.5% for solids) is added:

the amount of dye that penetrates the cornea:

**Step 4** Look at Figure 1 at right showing the structure of the eye. The eye consists of many different parts, each of which contributes to sight in different ways. For example, the pupil controls the amount of light that enters the eye (when there is less light, the pupil is larger; when there is more light, the pupil is smaller). Pupil size also changes when we focus on something close versus far away. The lens helps focus the image onto the retina, which collects the light. The retina and its associated neurons connect to the optic nerve, which carries the information to the brain for processing and interpretation of the image.

Consider the entire eye and how it functions, as well as the different routes of exposure for a chemical (i.e., a corrosive chemical that damages the surface layers versus a chemical that can be absorbed, ingested, or inhaled). What are some potential limitations of the BCOP assay with respect to testing "new products for their potential to cause temporary or permanent blindness, irritation, or other eye injuries"?

**Figure 1**




**Source:** National Eye Institute

Society of Toxicology

# Guiding Principles in the Use of Animals in Toxicology

1. The use, care, and transportation of animals for toxicological research, training, and testing for the purpose of protecting human and animal health and the environment must comply with all applicable animal welfare laws.

2. When scientifically appropriate, alternative procedures that reduce the number of animals used, refine the use of whole animals, or replace whole animals (e.g., *in vitro* models, invertebrate organisms) should be considered.

3. For research requiring the use of animals, the species should be carefully selected and the number of animals kept to the minimum required to achieve scientifically valid results.

4. All reasonable steps should be taken to avoid or minimize discomfort, distress, or pain of animals.

5. Appropriate aseptic technique, anesthesia, and postoperative analgesia should be provided if a surgical procedure is required. Muscle relaxants or paralytics are not to be used in place of anesthetics.

6. Care and handling of all animals used for research purposes must be directed by veterinarians or other individuals trained and experienced in the proper care, handling, and use of the species being maintained or studied. Veterinary care is to be provided in a timely manner when needed.

7. Investigators and other personnel shall be qualified and trained appropriately for conducting procedures on living animals, including training in the proper and humane care and use of laboratory animals.

8. Protocols involving the use of animals are to be reviewed and approved by an institutional animal care and use committee before being initiated. The composition and function of the committee shall be in compliance with applicable animal welfare laws, regulations, guidelines, and policies.

9. Euthanasia shall be conducted according to the most current guidelines of the American Veterinary Medical Association (AVMA) Panel on Euthanasia or similar bodies in different countries.